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UDC 575:633
DOI: 10.2298/GENSR1403719R
Original scientific paper

GENETIC DIVERSITY OF A SERBIAN GRAPEVINE GERMPLASM COLLECTION BASED ON MORPHOAGRONOMIC CHARACTERISTICS

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Rakonjac V., N.Korac, S.Todic, M.Medic, Z.Beslic, I.Kuljanic, D.Ivanisevic and M.Popov (2014): *Genetic diversity of a Serbian grapevine germplasm collection based on morphoagronomic characteristics*. - Genetika, Vol 46, No. 3, 719- 730.

The objective of this study was to evaluate diversity and relationships among grapevine cultivars from the Serbian gene bank in Sremski Karlovci, and to identify the most useful variables for discrimination. A total of 54 grapevine cultivars have been studied. During the period 2000–2005, twelve quantitative and qualitative characteristics were evaluated using OIV categories to each trait. Characterization was done using multivariate statistical analyses: cluster and principal component analysis (PCA), Based on cluster analysis cultivars divided into three major groups and the clustering pattern was related to the classical eco-geographical grouping: *occidentalis*, *pontica* and *orientalis*. Beside geographic origin clustering position of cultivars throughout the dendrogram was related to main uses. The major part (70.1%) of the total variation presented was explained by four principal components. PC1 is highly correlated with the bunch and berry size and PC2 with the density of prostrate hairs of young shoot tip and the density of prostrate hairs between veins on the lower side of mature leaf. The overall arrangement of cultivars suggests considerable phenotypical (and presumably genetic) variability in studied germplasm collection. Furthermore, obtained results may be useful for further utilization of available genetic resources in gene bank managers, growing and breeding.

Key words: amphelographic characteristics, cluster analysis, germplasm, PCA, *Vitis vinifera.*

INTRODUCTION

Due to intensive cultivation of a small number of commercial cultivars, there is a rapid loss of grapevine germplasm all over the world. The dominance of a small number of cultivars in world viticulture has resulted in an alarming reduction in genetic variability. Other existing cultivars are

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less exploited, and many have only a local significance or are largely confined to germplasm collections. The approximate number of different cultivars held in germplasm collections worldwide is 10,000 (ALLEWELDT and DETTWEILER, 1994; THIS *et al*., 2006). As a consequence of genetic erosion, we face the loss of cultivars the cultivation of which is traditionally related to different wine-growing regions (THIS *et al*., 2006; SANTANA *et al*., 2008; GAGO *et al*., 2009).

The only way to prevent the loss of this heritage is to have it located, studied and preserved in germplasm banks, so that it could be precisely characterized and correctly identified. Therefore, the preservation of grapevine germplasm is very important for breeding of new cultivars and for the preservation for future generations. The knowledge of genetic diversity and relationships among grapevine cultivars is important to recognize gene pools, identify differences in germplasm collections and developed effective conservation and management strategies. In addition, germplasm characterization and evaluation is a priority task for gen banks and a prerequisite to a successful breeding program (GOTOR *et al*., 2008). Morphological classifications provide useful guidelines to cultivar relations, developing further insight for plant breeders and gene bank managers. The first step in this study is to ampelographically described existing cultivars. Ampelographic evaluation of grapevine cultivars will enable the variety identification and facilitate the defining of the origin and genetic relationship among cultivars. Cultivars with a description and a known origin are good bases for the conservation of grapevine germplasm.

Serbia is located in the central Balkans, at the crossroads between Asia and Europe, on the line dividing various nations and civilizations of the East and the West. Due to its favourable climate and geological characteristics, the area of the Balkans is an ancient wine-growing region and both Serbia and many neighbouring countries have a long viticulture tradition (BEŠLIĆ *et al*.,2012). Many grapevine cultivars have been preserved and adopted in the Balkan region during the long grapevine migration history from Asia Minor to Europe. At the beginning of 2nd millennium B.C. domesticated grapevines were found in the Southern Balkan (KROLL, 1991). Balkan countries such as Serbia, Bulgaria, Montenegro, Bosnia and Herzegovina Macedonia, Greece, Turkey have a very long tradition in viticulture and represent a very rich gen pool of grapevines. The Balkan grapevine gene pool is poorly investigated in terms of genetic analysis. There are many cultivars with the same name or different names for the same genotype; besides, parent relations have not been defined. The study of biodiversity within the existing cultivars is important in order to preserve germplasm, identify cultivars and plan breeding programs (FOSSATI *et al*., 2001).

The main objective of this paper is to evaluate characterization of the cultivars existing in the Serbian grapevine genetic collection resources by means of ampelographic analysis of morphological appearance and chemical composition of grapes, to detect associations among cultivars and to identify the most useful variables for discrimination.

MATERIALS AND METHODS

Fifty-four grapevine cultivars with different morphological and technological characteristics have been evaluated. Tables 1a and 1b summarizes the plant material, with their origin, berry skin color and main use, being all of them cultivars of *Vitis vinifera.* Cultivars are planted in the amphelographic collection «Sremski Karlovci» wich belongs to the Faculty of Agriculture of the University of Novi Sad. This collection comprises more than 470 accessions including a large number of European cultivars and also a number of rare cultivars native to the Balkans and the region of Asia.

Table 1a. Group of analyzed Vitis vinifera cultivars *

Cultivar name	Suposed origin	Original pedigree	Berry skin[a]	Use
Adakalka	Serbia		B	Table
Afuz ali	Lebanon		B	Table
Aramon	France	Gouais blanc x ?	N	Wine/table
Bagrina	Former YU		Rs	Wine
Bakator belyi	Former USSR		B	Wine
Bela breza	Former YU		B	Wine
Bela dinka	Former YU		B	Wine
Bela ranina	Slovakia		B	Wine
Bele kozije sise	Former YU		B	Table
Beli medenac	Hungary		B	Wine
Black rose	United States	(Damas rose x Black monukka) x Alphonse lavallee	N	Wine/table
Cabernet franc	France		N	Wine
Cabernet Sauvignon	France	Sauvignon blanc x Cabernet franc	N	Wine
Cardinal	United States	Ribier x Reine des vignes	Rs	Table
Carignan	France		N	Wine
Chaouch blanc	Turkey		B	Table
Chasselas blanc	France		B	Wine/table
Chasselas rouge	Switzerland		Rs	Wine/table
Drenak crveni	Former YU		N	Table
Flame tokay	Algeria		Rs	Wine
Gamay	France	Pinot noir x Gouais blanc	N	Wine
Grüner veltriner	Italy		B	Wine
Italia	Italy	Bicane X Muscat hamburg	B	Table
Kadarka kek	Hungary		N	Wine
Kharistvala kolkhuri	Georgia		N	Wine/table
Kreaca	Former YU	Gouais blanc	B	Wine
Limberger	Austria		N	Wine
Merlot	France	Magdaleine noir des charentes x Cabernet franc	N	Wine

*Used Vitis International Variety Catalogue (VIVC) http://www.vivc.de

[a]B=white, Rs= pink, G=grey-red, N=bluish-black

Table 1b. Group of analyzed Vitis vinifera cultivars *

Cultivar name	Suposed origin	Original pedigree	Berry skin[a]	Use
Muscat a petits grains blancs	Greece		B	Wine
Muscat Fleur d' Oranger	France	Muscat a petits grains blancs x Chasselas blanc	B	Wine/table
Muscat Hambourg	United Kingdom	Schiava grossa x Muscat of Alexandria	N	Table/wine
Nimrang krasnyi	Uzbekistan		Rs	Table
Pamid	Bulgaria		Rs	Wine
Pinot blanc	France	Pinot noir mutation	B	Wine
Pinot gris	France	Pinot noir mutation	G	Wine
Pinot noir	France		N	Wine
Portugieser blau	Austria		N	Wine
Prokupac	Former YU		N	Wine
Riesling	Germany	(Vitis silvestris x Traminer) ? x Gouais blanc	B	Wine
Riesling italico	France		B	Wine
Rkatsiteli	Georgia		B	Wine/table
Ružica	Former YU		N	Wine
Sauvignon	France		B	Wine
Seduša	Former YU		N	Wine
Semillon	France		B	Wine
Smederevka	Bulgaria	Gouais blanc x ?	B	Wine/table
Sultanina	Turkey		B	Table/raisin
Syrah	France	Mondeuse blanche x Dureza	N	Wine
Szeremi zoeld	Hungary		B	Wine
Taifi rozovyi	Uzbekistan		Rs	Table
Traminer rot	Italy		Rs	Wine
Vranac	Montenegro		N	Wine
Žilavka	Bosnia and Herzegowina		B	Wine
Zimsko belo	FYROM		B	Table

*Used Vitis International Variety Catalogue (VIVC) http://www.vivc.de
[a]B=white, Rs= pink, G=grey-red, N=bluish-black

This collection is located in Vojvodina, to the North of Serbia (latitude 45º 10´ N, longitude 20º 10´ E and altitude 110 m). It has a temperate - continental climate with the annual average air temperature of 11.7 °C and the seasonal average temperature of 17.3°C. The total annual rainfall averages 758 mm, with 353 mm of rainfall during the growing season. The vineyard soil type is a

eutric cambisol. The collection was planted in 1979, at vine spacing 3 x 1m. The training system is a single Guyot. Winter pruning was uniform for each vine - mix of canes and spurs (12+2).

During the period 2000–2005, twelve quantitative and qualitative characteristics were evaluated by means of OIV descriptor list (ANONYMOUS, 1997): Young shoot - density of prostrate hairs of tip (OIV 004); Mature leaf - size (OIV 065) and density of prostrate hairs between the veins on lower side (OIV 084); Bunch - size (OIV 202), density (OIV 204) and length of peduncle (OIV 206); Berry- size (OIV 220), shape (OIV 223) skin color (OIV 225); Bunch weight/ha - yield (OIV 504); Sugar content of must (OIV 505); Total acid content of must (OIV 506).

Statistical analysis was carried out using OIV categories to each trait. Cluster analysis performed by UPGMA method, using Euclidean distances, was applied to evaluate relations among cultivars. A principal component analysis (PCA) was performed in order to summarize manifold data in the first principal component containing the highest possible variability of data. To determine wich of the PCs accounted for the greatest amount of variation, for each trait, the eigenvalues of the 4 PCs were compared for each characteristic. Data analysis was conducted using the 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA).

RESULTS

A dendrogram of 54 grapevine cultivars was generated using the UPGMA clustering method. Cultivars were classified into three main clusters (Figure 1). The clustering position of cultivars throughout the dendrogram was basically related to eco-geographic origins and main use. The first cluster predominantly includes table cultivars; the second cluster comprises Central and East Europe wine cultivars and the third cluster West Europe wine cultivars.

The maximum Euclidean distance (d = 17.09) is noted between Pinot Gris and Kharistvala kolkhuri, while the minimum distance (d = 2.00) is obtained between Chasseles blanc and Chasseles roug and between Caberne franc and Merlo. A similar level of variability is observed within the cluster II (d = 8.40) and cluster III (d = 8.50) and somewhat higher in the cluster I (d = 10.22). At the cluster level, the first level of connection is established between cluster II and cluster III in the group of wine cultivars which are then, with a high distance level (d = 11.15) linked to cluster I (predominantly including table cultivars).

The first cluster includes 15 cultivars divided in two subgroups. The first subgroup (IA) comprises of 4 table and one vine cultivars characterized by very large, loose bunches and large berries. The second subgroup (IB) consists of 9 cultivars characterized by medium large to large bunches and berries. Seedless cultivar Sultanina has proved to be the most distant from other cultivars of this cluster and it separates independently.

Cluster II is the largest and the most complex, and it encompass 24 cultivars. It is could be additionally divided into three subgroups. The first subgroup (IIA) includes 8 mainly wine cultivars intended for red wine, which have dark red or blue-black color of berry skin. The second subgroup (IIB) consists of two table cultivars Chaouch blanc and Drenak crveni. Fourteen cultivars with berry skin of predominantly green-yellow color are classified within the third subgroup (IIC).

Cluster III contains wine grapes originating from Western Europe divided into two subgroups primarily according to berry skin color. The first subgroup (IIIA) includes 6 red wine cultivars while the other subgroup (IIIB) includes 7 white wine cultivars.

Cultivar Kharistvala kolkhuri has been completely out grouped and initially separated from all the cultivars and it appears to be misnomer.

PCA used to identify the most significant variables in the data set produced four principal components with eigenvalues higher than 1 (Table 2). These components are enough to explain 70.1% of the total variability observed, with PC1, PC2, PC3 and PC4 accounting for 33.04%, 17.00%, 11.39% and 8.88% on the variance, respectively. This value of the extracted information is absolutely adequate considering the number of involved variables and the study's purpose.

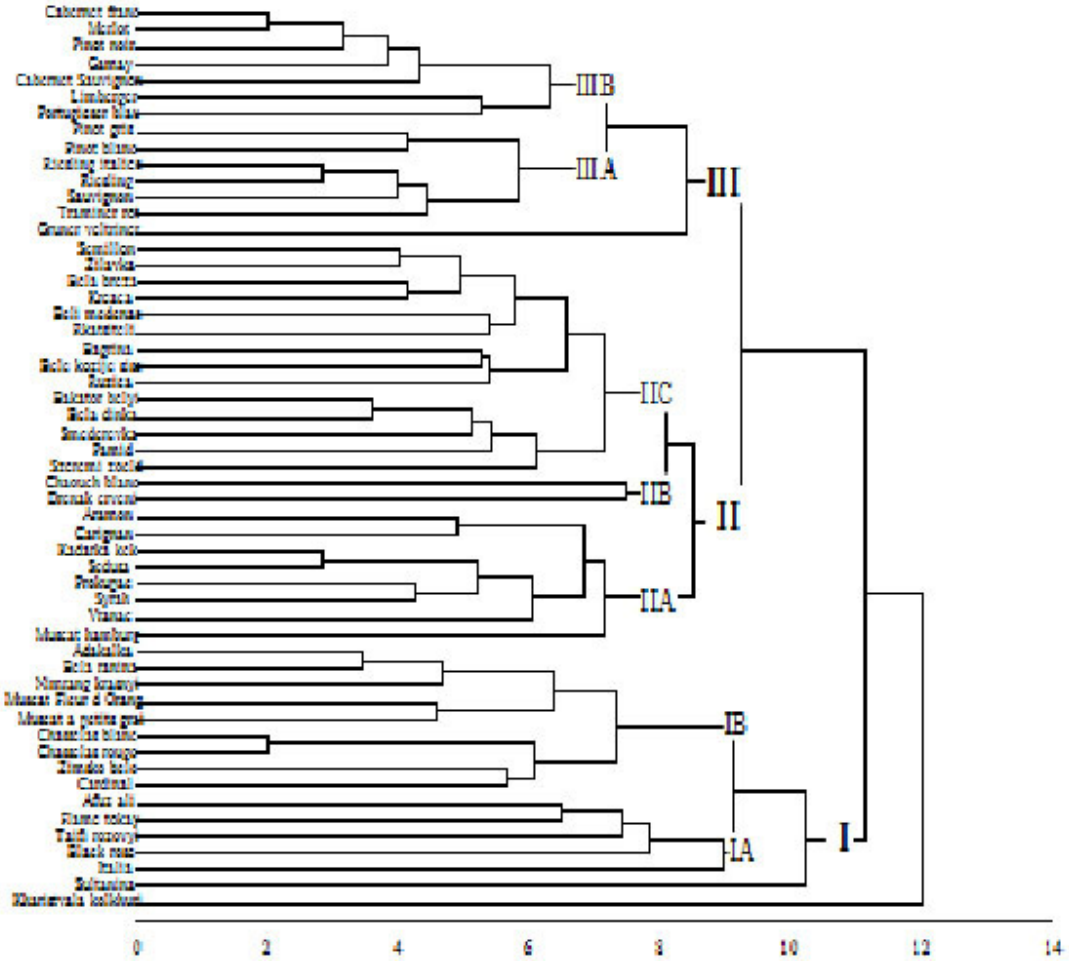

Figure 1. Cluster dendrogram of 54 grapevine cultivars obtained by the UPGMA, using 12 amphelographical and technological characteristics.

Table 2. Eigenvalues, proportion of total variability and correlation between the original variables and the first four principal components.

Variable[a]	PC1	PC2	PC3	PC4
OIV 004	0.465	0.766	0.251	0.102
OIV 065	-0.358	0.474	-0.054	0.087
OIV 084	0.224	0.872	0.295	0.018
OIV 202	-0.890	0.140	0.005	0.046
OIV 206	-0.791	-0.050	0.076	0.075
OIV 204	0.659	0.094	-0.357	0.199
OIV 220	-0.855	0.045	-0.028	0.155
OIV 223	-0.637	-0.167	0.196	0.155
OIV 225	0.004	0.043	-0.240	0.929
OIV 504	-0.364	0.513	-0.408	-0.202
OIV 505	0.657	-0.374	0.243	0.182
OIV 506	-0.126	-0.049	0.871	0.125
Eigenvalue	3.965	2.040	1.366	1.065
% Var.	33.04	17.00	11.39	8.88
% Cum.	33.04	50.04	61.43	70.31

[a] For OIV codes see *Materials and methods*

Correlation between the original variables and the first 4 principal components is shown in Table 2. Variables with higher scores on PC1 (over 0.70 of absolute value) are bunch and berry size and length of peduncle. The highest contribution of PC2 corresponded to variables related to density of prostrate hairs of shoot tip and density of prostrate hairs between veins on lower side of blade of mature leaf. High loading on PC3 had the total acid content of must. Finally, the largest scores of PC4 were due to berry color.

DISSCUSSION

The 54 cultivars of the «Sremski Karlovci» collection studied, originate from more countries around the world, making our sample representative of the cultivated grapevine gene pool. Our data confirmed the high levels of diversity of the cultivated grapevine, in agreement with previous studies (ARADHYA *et al.*, 2003; THIS *et al.,* 2006; LAUCOU *et al*., 2011; STAJNER *et al*., 2014). Also, our results shown that traditional descriptive methods, based on plant vegetative and reproductive traits, have greatly contributed to establishing the identity of cultivars because each cultivar has had a unique combination of scores for the characteristics studied. This is in agreement with ORTIZ *et al*., (2004) who cited that morphological characterization of grapevine cultivars necessary in order to describe, from a practical viewpoint, and it is also have great importance for the characterization of accessions in gene banks.

Since phenotypic characterization involves a wide range of data which include both qualitative and quantitative traits, with a considerable number of descriptors measured for each of many genotypes application of multivariate statistics is recommended. The most widely used multidimensional analysis methods in genetic studies are Principal component analysis (PCA) and cluster analysis (CA) (RAKONJAC *et al*, 2010; DJORDJEVIĆ *et al*., 2014)

On the dendrogram obtained by cluster analysis cultivars are randomly mixed and clustered into group and subgroup, which suggests their dissimilarity. Separation of cultivars in clusters primarily was a function of it eco-geographic origin. Previously, on the bases to geographical origin Negrul, spotted that grapevine cultivars greatly differed in numerous morphological and biological characteristics; therefore he has defined three groups (convarietas, proles): *occidentalis, orientalis* and *pontica* (ARADHYA *et al.,* 2003). In according with these three major clusters I, II, and III revealed by our study, were designated as *orientalis, pontica and occidentalis,* respectively. Other authors (BISSON, 1995; ARADHYA *et al.,* 2003; GUO *et al.,* 2010; GOTO-YAMAMOTO *et al.,* 2006; BACILIERI *et al*., 2013) also noted the separation of grape cultivars in three eco-geographic groups, either based on ampelographic description or based on molecular markers. Expect the origin the use of grapes was an important criterion for cultivars distributions in clusters, which is in agreement with HEUERTZ *et al*., (2008). The first cluster predominantly includes table cultivars; while the second and the third cluster comprises wine cultivars.

The level of variability observed within three main clusters in this paper is in agreement with results of GUO *et al*., (2010) who found out that oriental cultivars show a greater degree of genetic difference from *occidental* and *pontica* cultivars within the species *Vitis vinifera*. This is consistent with the hypothesis that grapevine domestication initially occurred in Eastern regions (THIS *et al*., 2006) where it is further spread.

The first cluster includes mainly table cultivars possessed morphological traits distinctive of group *orientalis*. These traits are: the absence of sparsely distributed prostrate hairs on shoot tips and non-prostrate hairs on the lower side of mature leaf. Apart from typical table cultivars, this subgroup encompasses certain wine cultivars as Muscat a petits grains blancs, Chassales rouge Chassales blanc, and Fleur d' Oranger. Muscat a petits grain blancs originates from Greece, and it is considered a typical representative of convar. *orientalis*. There are certain doubts about the origin of Chassales varieties. Legends have them associated with the Near East or with Western Europe, according to some other sources (VOUILLAMOZ and ARNOLD, 2009). The fact that Muscat Fleur d' Oranger falls within this group could be explained by its French origin and its creation through the crossing of Muscat a petits grains blancs x Chasselas blanc, which fall within this cluster as well.

Most cultivars of cluster II are wine or dual use ones, of Balkan or Eastern Europe origins. They are characterized by dense to very dense prostrate hairs on young shoot tip and lower side of mature leaf, medium to large bunch and berry size related to *pontica*. Cluster II also encompasses certain Western European wine cultivars such as Carignan, Aramon, Syrah and Semillion and table cultivar Muscat Hamburg, which should morphologically (hairiness of leaves and young shoot tip and bunch and berry size) fall within convar. *pontica* rather than convar. *occidentalis*. This is in agreement with the results of ARADHYA *et al*., (2003) who have applied NJ and UPGMA cluster analysis based on eight microsatellite loci and also found out that Carignan, Muscat Hamburg and Aramon belong to group *pontica*.

Cluster III contains wine grapes originating from Western Europe. Cultivars of this cluster are characterized by weak to medium density of prostrate hairs between the veins on the

lower leaf side, small compact benches and very small to small berries. According to LEVADOUX (1956) these cultivars have numerous characteristics of wild grapes; hence they could be representatives of the autochthonous form *Vitis vinifera.* The cultivars of *occidentalis* group have been divided into two subgroups primarily according to berry skin color which in agreement to DHANORKAR *et al.,* (2005).

Overall results of cluster analysis indicate that *orientalis* and *pontica* cultivars are grouped according to their origin, while Western European varieties are present in all three separate groups. This is supported by AMBROSI *et al*., (1994) who consider present-day European grape genes pools are made of the cultivars domesticated from wild indigenous vines, as well as through introduction and introgression of the Near Eastern germplasm. This tendency supports the belief that the spread of viticulture from Eastern to Western Europe has involved dissemination of domesticated varieties in different times (BOUQUET, 1982).

A high correlation been noted between certain traits and principal components, which could reduce the number of traits to be studied in grapevine germplasm evaluation. Those are traits related to hairines, berry skin color, bunch and berry size and total acid content of must. At the same time, these characteristics are the most important in agricultural practice and breeding. The results correspond to LEÃO *et al*., (2010, 2011) who applied PCA to wine and table grapes and found out that most of the variability was retained in the first four components. High absolute values of correlations between variables related to bunch and berry size, and PC1 or PC2 in different grape germplasm collection were also established by FATAHI *et al*., (2004) and MATTHEOU *et al*., (1995a,b).). Also, RAKONJAC *et al.,* (2010) found the grape weight had the most influence on the Kreaca clones separation in groups.

PCA results indicate that the observed variability in the studied grapevine collection has been equally influenced by quantitative and qualitative characteristics. However, the dendrogram shows that properties such as hairiness of leaves and young shoot tips and berry color, i.e. monogenic qualitative traits have most considerably influenced the classification of cultivars into groups and subgroups. Then follow bunch and berry size, and finally the content of acids, which fall within the category of quantitative traits. If, apart from the above, we also take into account the fact that quantitative traits, besides genotype, are influenced by environmental factors, qualitative characteristics could be suggested as more reliable for germplasm characterization.

As a conclusion, a grapevine cultivars description makes it easier to identify interesting properties and to ensure that the whole spectrum of variation is preserved. Overall organization of diversity suggests that minimum gains in the variability are possible if we collect from diverse eco-geographic sources, since its structure is determined by artificial selection and a vegetative mode of reproduction. For increasing genetic variability in grapevine germplasm hybridization of cultivars from different eco-geographical group or interspecies can be recommended.

ACKNOWLEDGEMENTS

The authors wish to thank the Ministry of Education and Science of the Republic of Serbia for their financial support of this research, as a part of the projects TR 31063 and III 43007.

Received March 17th, 2014

Accepted September 05th, 2014

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GENITIČKA VARIJABILNOST SRPSKE GERMPLAZME VINOVE LOZE NA OSNOVU MORFOAGRONOMSKIH KARAKTERISTIKA

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Izvod

Cilj istrazivanja je bio da se proceni diverzitet i odrede relacije između sorti vinove loze koje se nalaze u srpskoj kolekciji u okviru gen banke u Sremskim Karlovcima i da se izdvoje osobine koje su imale najveći uticaj na njihovo grupisanje. U periodu od 2000 do 2005. godine proučavano je 12 kvantitativnih i kvalitativnih osobina kod 54 sorte vinove loze različitog porekla i namene. Osobine su utvrđene primenom OIV deskriptora i iskazane poentiranjem. Morfološka karakterizacija sorti je izvršena primenom multivarijacionih statističkih analiza: klaster i PCA (Principal Component Analysis). Primenom hijerarhijske klaster analize, sorte su se svrstale u tri osnovne grupe (*occidentalis*, *pontica* i *orientalis)* što se podudara sa ekološko geografskim klasifikacijom sorti vinove loze. Pored geografskog porekla, na razvrstavanje sorti pre svega u podklastere veliki uticaj je imala i njihova namena. Analiza glavnih komponenti (PCA) je pokazala da prve četiri PC objašnjavaju najveći deo uočene varijabilnosti (71%) pri čemu je PC1 bila visoko korelisana sa veličinom grozda i bobice a PC2 sa gustinom malja na vrhu mladog lastara i gustinom malja između nerava na naličju lista. Način povezivanja sorti na dendrogramu ukazuje na postojanje značajne fenotipske (a samim tim i genetičke) varijabilnosti u proučavanoj kolekciji germplazme. Dobijeni rezultati o raspoloživoj germplazmi mogu biti značajni za korišćenje u svrhe menadžmenta gen banke, pri izboru sorti za gajenje ili pri daljem oplemenjivačkom radu.

Primljeno 17. III. 2014.
Odobreno 05. IX. 2014.